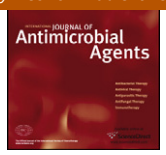
International Journal of Antimicrobial Agents 38 (2011) 307–313

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag



Pharmacokinetic/pharmacodynamic modelling of the bactericidal activity of free lung concentrations of levofloxacin and gatifloxacin against *Streptococcus pneumoniae*

Leandro Tasso[a,1], Cristiane de Andrade[b], Teresa Dalla Costa[a,b,*]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, Porto Alegre, RS, 90.610-000, Brazil*
[b] *Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 24 February 2011
Accepted 12 May 2011

Keywords:
Pharmacokinetic/pharmacodynamic modelling
Levofloxacin
Gatifloxacin
In vitro infection model

ABSTRACT

The aim of this work was to compare the pharmacological properties of levofloxacin and gatifloxacin against *Streptococcus pneumoniae* by pharmacokinetic/pharmacodynamic (PK/PD) modelling of the time–kill curves employing an E_{max} model. An in vitro infection model was used to simulate free pulmonary fluctuating concentrations expected after multiple dosing regimens of both drugs in humans or constant multiples of the minimum inhibitory concentration. PK/PD parameters and PK/PD indices of the simulated dosing regimens were compared. The levofloxacin EC_{50} (concentration producing 50% of the maximum effect) (mean ± standard deviation 3.57 ± 2.16 mg/L) was higher than that for gatifloxacin (0.95 ± 0.56 mg/L) when simulating multiple dosing regimens as well as constant concentrations ($EC_{50,levofloxacin}$ = 2.75 ± 0.45 mg/L; $EC_{50,gatifloxacin}$ = 1.03 ± 0.52 mg/L). The maximum killing rate constant (k_{max}) was not statistically different for both drugs independent of fluctuating ($k_{max,levofloxacin}$ = 0.40 ± 0.19 h^{-1}; $k_{max,gatifloxacin}$ = 0.48 ± 0.15 h^{-1}) or constant concentrations ($k_{max,levofloxacin}$ = 0.34 ± 0.06 h^{-1}; $k_{max,gatifloxacin}$ = 0.39 ± 0.23 h^{-1}). The PK/PD model was able to describe the effect of levofloxacin and gatifloxacin against *S. pneumoniae* in vitro for all the simulations investigated. Gatifloxacin was more potent than levofloxacin, but both presented equivalent efficacy. The model can be used for simulating alternative regimens and optimising therapy to treat streptococcal infextions.

1. Introduction

Although improvements in antibiotic and supportive treatment have occurred in recent years, community-acquired pneumonia (CAP) is an increasingly common reason for hospital admission and still remains a major cause of both mortality and morbidity worldwide [1]. Several factors, including extreme age and co-morbidities, contribute to its high mortality risk [2,3].

Despite the emergence of newly identified pathogens related to CAP, *Streptococcus pneumoniae* remains the most common aetiological agent [4,5]. Respiratory fluoroquinolones such as levofloxacin and gatifloxacin are effective antimicrobial agents indicated for the treatment of lower respiratory infections, particularly CAP [6]. Levofloxacin and gatifloxacin are also used for the treatment of chronic bronchitis as well as urinary tract, kidney and skin infections [7,8]. Both drugs have a broad spectrum of antimicrobial activity against Gram-negative and Gram-positive pathogens [9].

Antimicrobial agents are evaluated based on pharmacokinetic/pharmacodynamic (PK/PD) indices. Using PK/PD indices, antimicrobial activity can be classified as time-dependent, i.e. the key factor that correlates with efficacy is the percentage of time that the antimicrobial concentration remains above the minimum inhibitory concentration of the pathogen (T > MIC), or as concentration-dependent, when the ratio between antimicrobial free peak plasma level (fC_{max}) and MIC (fC_{max}/MIC) or between area under the free plasma concentration–time curve (fAUC) and MIC (fAUC/MIC) better forecast bacterial eradication [10]. Although both fC_{max}/MIC and fAUC$_{0-24h}$/MIC correlate with successful therapeutic outcomes for fluoroquinolones, studies advocate that the fAUC$_{0-24h}$/MIC ratio is the best index to predict the therapeutic outcome of fluoroquinolones [11,12]. Data from in vitro, animal and clinical studies suggest that an fAUC$_{0-24h}$/MIC ratio between 25 and 34 is the target for fluoroquinolones to permit bacterial eradication in CAP caused by *S. pneumoniae* [13]. However, other authors suggest that for CAP due to this microorganism, an fAUC$_{0-24h}$/MIC ratio ≥30 is predictive of infection outcome [14–19].

* Corresponding author. Tel.: +55 51 3308 5418; fax: +55 51 3308 5437.
E-mail address: teresadc@farmacia.ufrgs.br (T. Dalla Costa).
[1] Present address: Faculty of Pharmacy, Universidade de Caxias do Sul, Caxias do Sul, RS, Brazil.

0924-8579 © 2011 Elsevier B.V. and the International Society of Chemotherapy. Open access under the Elsevier OA license.
doi:10.1016/j.ijantimicag.2011.05.015

Although the MIC is a good PD parameter to describe the antibacterial potency of a drug, it is a threshold concentration with poor precision, determined under static in vitro conditions. In vivo, bacteria are exposed to fluctuating peak and trough concentrations and the antibacterial activity is a result of a dynamic concentration- and time-dependent process [20]. Consequently, PK/PD indices that use the MIC are limited by the same boundaries. Furthermore, PK/PD indices define target values that ensure bacterial eradication, not allowing for comparisons between different dosing regimens of the same drug, efficacy comparison of different drugs used to treat the same infection, or outcome prediction for alternative regimens.

Aiming to overcome these limitations, in PK/PD modelling the PD effect determined in time–kill experiments is modelled taking into consideration the pharmacokinetics of the antimicrobial at the infection site. As a result, a mathematical description of the antimicrobial effect–time profile is obtained and pharmacologically meaningful parameters are derived. These parameters allow not only to describe the antibacterial behaviour of the drug under investigation but also to compare drugs of the same class for treating a specific infection as well as to simulate the effect over time of alternative regimens.

Considering that most infections occur in peripheral tissues and not in the bloodstream, and that only free drug concentrations exhibit a pharmacological effect, antimicrobial free tissue levels should be used in PK/PD modelling. In general, fluoroquinolones have extensive tissue penetration, showing $AUC_{free,tissue}/AUC_{free,plasma}$ ratios close to 1. Levofloxacin showed ratios between 0.85 in skeletal muscle [21] and 1.1 in subcutaneous adipose tissue [22] in humans. Free pulmonary concentrations of levofloxacin have been determined by microdialysis in healthy individuals following cardiac surgery [23]. Using the data presented in this paper, levofloxacin estimated pulmonary penetration was determined to be 0.82. Our group previously demonstrated that gatifloxacin easily penetrates into pulmonary Wistar rat tissue [24]. Free pulmonary levels of the drug could be predicted using PK parameters derived from total plasma concentrations and were similar to free plasma levels. The lung penetration factor was found to be 1.08.

The purpose of this study was to compare pharmacologically the effects of levofloxacin and gatifloxacin against *S. pneumoniae* by PK/PD modelling of the time–kill curves obtained using an in vitro experimental infection model where the expected lung free levels of both drugs following different dosing regimens in humans were simulated.

2. Materials and methods

2.1. Bacterial strains and growth media

Streptococcus pneumoniae ATCC 49619 was kept frozen at −70 °C in sterile skim milk (Acumedia, Lancing, MI) with a drop of sterile sheep blood. For the experiments, the strain was transferred to Mueller–Hinton agar (MHA) (Difco, Detroit, MI) plates supplemented with 5% sheep blood (McBarth, Porto Alegre, Brazil) and was incubated for 18–24 h at 35 ± 1 °C in a CO_2 atmosphere. For the inoculum preparation, isolated colonies were re-suspended in sterile saline to reach a turbidity equivalent to a 0.5 McFarland standard.

2.2. Antimicrobials and susceptibility testing

Levofloxacin (99.7%) and gatifloxacin (100%) were donated by Cristalia and Bristol-Myers Squibb (São Paulo, Brazil), respectively. MICs of levofloxacin and gatifloxacin were determined in triplicate using the broth macrodilution method according to procedures outlined by the Clinical and Laboratory Standards Institute (CLSI) [25].

2.3. In vitro model of infection

A simple one-compartment in vitro model was used to study the PD effect of fluoroquinolones against *S. pneumoniae* following multiple intermittent dosing of the drugs or constant concentrations. The in vitro model consisted of a 50 mL culture flask containing 20 mL of Todd–Hewitt broth (Difco, Sparks, MD). The medium was prepared according to the manufacturer's instructions and was autoclaved at 121 °C prior to use. Withdrawal and replacement of broth solution for the simulation of decreasing concentrations were performed at fixed time intervals using a sterile 0.22 μm filter, and the bacterial count was kept unchanged in the medium. An aliquot of the *S. pneumoniae* suspension [10^8 colony-forming units (CFU)/mL] was added to the system to achieve a consistent starting inoculum of ca. 5×10^5 CFU/mL.

To ensure that bacteria were in logarithmic growth phase prior to antimicrobial exposure, experiments were previously performed to determine the lag time. After 2 h of incubation, a time zero sample was taken and the drug was added to the flask. Bacteria were exposed to different initial concentrations of both fluoroquinolones corresponding to the expected free peak levels of the drugs in pulmonary tissue. To reproduce the half-life of each drug, a stepwise dilution procedure was followed. At 30-min intervals, aliquots of the experimental media containing the drugs were withdrawn and were replaced by drug-free sterile broth solution at 35 ± 1 °C during 24 h of experiment. In another set of experiments, bacteria were exposed to different constant concentrations of both drugs and the kill curves were built up to 12 h.

To assess bacterial density over time, samples were collected every 2 h up to the end of the experiment and were serially diluted 10-fold in sterile normal saline. Two experiments were followed up to 4 h collecting samples every 30 min [levofloxacin 1000 mg every 24 h (q24h) and gatifloxacin 200 mg q24h]. Aliquots of each diluted sample were plated in duplicate. All experiments were conducted in triplicate. Together with each treatment, one control experiment was carried out to assure bacterial growth in the in vitro model.

Following 18–24 h of incubation on MHA plates supplemented with 5% sheep blood, the number of CFU/mL in the samples was determined. The lowest level of detection was 200 CFU/mL. Time–kill curves were constructed by plotting $\log_{10}$ CFU/mL against time. Logarithmic reductions in bacterial counts were determined by subtracting 24 h CFU/mL values from the starting inoculum.

2.4. Pharmacokinetics of fluoroquinolones in the in vitro infection model

To simulate the pharmacokinetics in the in vitro infection model, drugs were added to the experimental flask aiming to reach the free pulmonary peak concentration (fC_{max}) expected for each drug regimen simulated.

Levofloxacin expected free concentrations in lung were calculated based on the experiments conducted by Hutschala et al. [23] using microdialysis in patients who received conventional 500 mg once-daily dosing of the drug. Free lung levels of levofloxacin in this study showed elimination following two slopes, with 30 min and 7 h half-lives, respectively. To simulate this elimination profile in vitro, in the first step-dilution 10 mL of media was withdrawn and replaced by fresh broth. In the following steps, 0.97 mL of the medium was exchanged by fresh broth.

Levofloxacin free levels in the in vitro infection model were described by Eq. (1):

$$C = ae^{-\alpha t} + be^{-\beta t} \quad (1)$$

Table 1
Pharmacokinetic/pharmacodynamic (PK/PD) indices associated with the simulated dosage regimens for levofloxacin and gatifloxacin, and PK/PD parameters determined by modelling the killing effect of levofloxacin and gatifloxacin against *Streptococcus pneumoniae* to an E_{max} model with fluctuating concentrations.

Simulated dose regimen	Daily dose (mg)	fC_{max} (mg/L)	fC_{max}/MIC	$fAUC_{0-24h}$ (mg h/L)	$fAUC_{0-24h}$/MIC (h)	EC_{50} (mg/L)	k_{max} (h^{-1})	MSC (R^2)
Levofloxacin (MIC = 1.0 mg/L)								
250 mg q24h	250	3.2	3.2	15	15	7.14	0.70	3.0 (0.97)
167 mg q8h	500	2.2	2.2	30	30	4.98	0.70	3.7 (0.97)
250 mg q12h	500	3.2	3.2	30	30	2.83	0.30	2.8 (0.90)
500 mg q24h	500	6.4	6.4	30	30	5.71	0.37	2.3 (0.90)
375 mg q12h	750	4.9	4.9	45	45	3.00	0.32	3.7 (0.97)
750 mg q24h	750	9.8	9.8	45	45	2.20	0.30	5.0 (0.99)
500 mg q12h	1000	6.4	6.4	60	60	0.87	0.17	4.6 (0.99)
1000 mg q24h	1000	12.8	12.8	60	60	1.79	0.35	5.1 (0.99)
Mean ± S.D.						3.57 ± 2.16*	0.40 ± 0.19	
Gatifloxacin (MIC = 0.5 mg/L)								
50 mg q24h	50	0.35	0.7	3.4	6.8	0.57	0.42	2.9 (0.92)
50 mg q12h	100	0.35	0.7	6.8	13.6	0.38	0.3	3.6 (0.96)
100 mg q24h	100	0.7	1.4	6.8	13.6	1.35	0.57	4.4 (0.98)
200 mg q24h	200	1.4	2.8	13.6	27.2	1.50	0.64	4.3 (0.99)
Mean ± S.D.						0.95 ± 0.56*	0.48 ± 0.15	

fC_{max}, free peak concentrations in plasma; MIC, minimum inhibitory concentration; fAUC, area under the free plasma concentration–time curve; EC_{50}, concentration producing 50% of the maximum effect; k_{max}, maximum killing rate constant; MSC, model selection criterion; q24h, every 24 h; q8h, every 8 h; q12h, every 12 h; S.D., standard deviation.
* Statistical difference ($\alpha = 0.05$).

where C is the expected free lung concentration in humans at any time t, a and b are the intercepts for the first and second elimination rates, respectively, and α and β are the constant rates representing the first and the second elimination, respectively. The values of the hybrid constants a, b, α and β were calculated based on the free pulmonary data reported for the 500 mg dose [23]. Assuming linear pharmacokinetics, peak levels for the other dosing regimens were calculated.

Gatifloxacin free peak pulmonary levels in humans were assumed to be similar to free plasma levels, based on the drug tissue penetration experiments conducted by our group [24], and were determined using plasma concentrations reported by Zhang et al. [26] corrected by a protein binding of 20% [27]. An elimination half-life of 7.5 h was simulated.

Gatifloxacin free levels in the in vitro infection model were described by Eq. (2):

$$C = C_{max} e^{-ket} \quad (2)$$

where C_{max} is the peak free concentration after dosing and ke is the elimination rate constant.

The dosing regimens simulated and the corresponding free peak concentrations of each drug added to the in vitro model are shown in Table 1, as well as the PK/PD indices calculated. The AUC over 24 h (AUC_{0-24h}) for both fluoroquinolones was determined by trapezoidal rule.

For the experiments where constant concentrations were simulated, both drugs were added to the infection model at levels corresponding to multiples of the MIC and were kept constant through the experiments (in triplicate). The same procedures described for fluctuating concentrations were employed to determine the CFU/mL counts every 2 h up to 12 h.

2.5. Determination of antimicrobial concentrations in the in vitro infection model

To assure that the stepwise dilutions used in the in vitro infection model closely resemble the elimination kinetics of both drugs expected in humans, samples of broth taken from each treatment were assayed. A high-performance liquid chromatography (HPLC) method was developed and validated to analyse these samples. The system employed a reversed-phase Shim-Pack CLC-ODS column (Chiyoda-Ku, Tokyo, Japan) and fluorescence detection (295 nm excitation wavelength and 480 nm extinction wavelength). The mobile phase consisted of 2.5 mM phosphoric acid:methanol:acetonitrile:triethylamine (64.8:15:20:0.2, v/v/v/v) at a flow rate of 1.0 mL/min. Proteins present in broth samples were precipitated with methanol. Samples were vortexed, centrifuged (6800 × *g*, 21 °C, 12 min) and the supernatants were injected in the Shimadzu® HPLC system (Chiyoda-Ku). Levofloxacin was used as an internal standard when gatifloxacin was evaluated, and vice versa.

The levofloxacin assay was linear ($r \geq 0.9987$) over a concentration range of 0.25 mg/L to 6.0 mg/L. The intra-assay and inter-assay precision for the 0.6, 1.5 and 4.5 mg/mL quality control samples did not exceed 1.08%, 1.01% and 0.48%, with 1.99%, 2.01% and 3.23% coefficient of variation (CV), respectively. The gatifloxacin assay was linear ($r \geq 0.9988$) over a concentration range of 0.15 mg/L to 3.0 mg/L. The intraday and interday precision for the 0.4, 1.2 and 2.5 mg/mL quality control samples did not exceed 2.06%, 1.35% and 4.21%, with 1.1%, 0.57% and 1.63% CV, respectively.

2.6. Pharmacodynamic data analysis

The experimental mean effect for each dosing regimen investigated was fitted to an E_{max} (sigmoidal effect/concentration model) model using the non-linear regression software Scientist® v. 2.01 (MicroMath Inc., Salt Lake City, UT):

$$\frac{dN}{dt} = \left(k_0 - \frac{k_{max} C}{EC_{50} + C}\right) N \quad (3)$$

where dN/dt is the change in the number of bacteria as a function of time, k_0 (h^{-1}) is the bacterial growth rate constant in the absence of antibiotic, k_{max} (h^{-1}) is the maximum killing rate constant, EC_{50} (mg/L) is the concentration of antimicrobial necessary to produce 50% of the maximum effect, C (mg/L) is the concentration of drug at any time (t) and N (CFU/mL) is the number of viable bacteria at the beginning of the experiment. For the experiments simulating fluctuating levofloxacin and gatifloxacin free pulmonary levels, the term C was replaced by Eqs. (1) and (2), respectively. For the constant concentration simulations, the term C was kept constant at a level corresponding to each multiple of the MIC investigated.

For the control experiment, bacterial growth was fitted to Equation 4 in which the variation in the number of bacteria in the infection model (dN/dt) is a function only of the bacterial growth rate constant (k_0) and the inoculum (N):

$$\frac{dN}{dt} = k_0 N \quad (4)$$

The k_0 was determined by simultaneous fitting of all control experiments conducted. When fitting the killing curves, k_0 was

fixed at 0.057 h^{-1} and the other parameters (EC_{50} and k_{max}) were estimated individually for each dosing regimen investigated.

The coefficient of determination (R^2) and the model selection criterion (MSC), given by the software Scientist®, as well as the correlation between observed and calculated values were used as criteria for the goodness of fit. No weighting factor was used to fit the data sets to the E_{max} model. The PK/PD parameters determined by modelling the killing effect of both drugs were compared by Student's *t*-test ($\alpha = 0.05$).

3. Results

The MIC for levofloxacin (1.0 mg/L) against *S. pneumoniae* ATCC 49619 was two-fold that determined for gatifloxacin (0.5 mg/L), in agreement with previously reported values [25]. The half-lives determined experimentally for levofloxacin (6.9 ± 0.1 h) and gatifloxacin (7.4 ± 0.1 h) were in agreement with the values simulated, confirming that the stepwise dilutions were adequate to mimic the fluctuating free concentrations expected in lung.

Streptococcus pneumoniae time–kill curves after multiple dosing are illustrated in Figs. 1 and 2 for levofloxacin and gatifloxacin, respectively. Fig. 3 shows the time–kill curves when constant concentrations were simulated. All levofloxacin multiple-dose regimens produced bacterial killing during the initial 8 h of treatment, with different intensity depending on the regimen investigated. For gatifloxacin, bacterial killing was observed during the initial 16 h following multiple dosing.

A 3-log reduction (99.9%) in the number of *S. pneumoniae* in the medium was observed for all treatments simulated except for levofloxacin 250 mg every 12 h (q12) and q24h, levofloxacin 500 mg q24h and gatifloxacin 50 mg q24h. For constant concentrations, a 3-log reduction in CFU was observed for levofloxacin and gatifloxacin when concentrations similar to MIC and 2× MIC were evaluated.

The PK/PD parameters obtained by modelling bactericidal effect to fluctuating and constant concentrations are presented in Tables 1 and 2, respectively. The PK/PD indices calculated for each dosing regimen investigated for both fluoroquinolones are also presented in Table 1.

It can be observed in Figs. 1–3 that the PK/PD model adequately described both fluoroquinolones' antimicrobial effect, with MSC values for the curve fitting greater than 2.3. The k_{max} determined for both drugs using different regimens was statistically similar ($\alpha = 0.05$) (Tables 1 and 2). The mean ± standard deviation EC_{50} determined for levofloxacin (3.57 ± 2.16 mg/L) was higher than that determined for gatifloxacin (0.95 ± 0.56 mg/L) when multiple-dosing was simulated in vitro (Table 1) as well as when constant concentrations were simulated (Table 2) where $EC_{50,levofloxacin}$ was 2.75 ± 0.45 mg/L and $EC_{50,gatifloxacin}$ was 1.03 ± 0.52 mg/L. Independent of the regimen investigated (constant or fluctuating concentration), the EC_{50} of each fluoroquinolone was statistically similar ($\alpha = 0.05$).

Table 2
Pharmacokinetic/pharmacodynamic (PK/PD) parameters determined by modelling the killing effect of constant concentrations of multiples of the minimum inhibitory concentration (MIC) of levofloxacin and gatifloxacin against *Streptococcus pneumoniae* to an E_{max} model.

Drugs and dosing regimens	EC_{50} (mg/L)	k_{max} (h^{-1})	MSC (R^2)
Levofloxacin (MIC = 1.0 mg/L)			
0.5× MIC	2.49	0.29	4.4 (0.99)
1× MIC	3.27	0.33	4.4 (0.99)
2× MIC	2.50	0.40	4.4 (0.99)
Average ± S.D.	2.75 ± 0.45*	0.34 ± 0.06	
Gatifloxacin (MIC = 0.5 mg/L)			
0.5× MIC	0.64	0.16	5.6 (0.98)
1× MIC	0.83	0.38	5.6 (0.99)
2× MIC	1.62	0.62	5.6 (0.99)
Average ± S.D.	1.03 ± 0.52*	0.39 ± 0.23	

EC_{50}, concentration producing 50% of the maximum effect; k_{max}, maximum killing rate constant; MSC, model selection criterion; S.D, standard deviation.
* Statistical difference ($\alpha = 0.05$).

4. Discussion

PK/PD modelling of the more realistic time–kill curves has the advantage of describing the PD effect by parameters such as EC_{50}, which describes the potency of the drug, and k_{max}, which represents drug efficacy, allowing for comparison of drugs used to treat a particular infection under similar experimental conditions.

The results obtained by fitting the experimental data to the E_{max} model showed that the model was adequate to describe the curves of antimicrobial effect for both drugs independently of the dosing regimen investigated (Figs. 1–3). The pharmacological effects of other fluoroquinolones such as ciprofloxacin [28,29] and norfloxacin [30] were already evaluated by similar E_{max} models.

Comparison of the PK/PD average parameters obtained from the time–kill curve fitting showed that both drugs were equally efficacious but that gatifloxacin was more potent than levofloxacin to treat *S. pneumoniae* infection when multiple-dose regimens or constant multiples of the MIC were simulated (Tables 1 and 2).

The PD effect of these quinolones can be viewed from a different perspective. When contrasting both drugs' dosing regimens with equivalent fC_{max}/MIC indices one can reach a different conclusion. Comparing, for instance, the regimens that produced fC_{max}/MIC close to 3 times the MIC for levofloxacin (250 mg q12h) and gatifloxacin (200 mg q24h) one can observe that the effect of gatifloxacin was more pronounced, showing bigger k_{max} values (Table 1; Figs. 1 and 2). Similar behaviour can be observed when constant multiples of the MIC were used. Comparing the time–kill curves for the equipotent concentration of 2× MIC (Fig. 3), for example, the number of CFU decreased faster for gatifloxacin than for levofloxacin, resulting in a bigger k_{max} (Table 2). The same can be observed for the other concentrations investigated. According to this observation, gatifloxacin appears to be more potent and efficacious than levofloxacin in eradicating infection produced by this bacterial strain.

Use of PK/PD indices to select the best dosing schedule for antimicrobial therapy is still one of the main approaches in clinical practice. When levofloxacin was first approved by the US Food and Drug Administration (FDA), the dosing regimen recommended was 500 mg q24h. Assuming that the $fAUC_{0-24h}$/MIC index is the best predictor of the drug effect, fractionating the total daily dose should not interfere with the final effect. In the same manner, if the fC_{max}/MIC was the best index, 500 mg once-daily would be more effective. The results observed in Fig. 1 for levofloxacin 167 mg every 8 h (Fig. 1A), 250 mg q12h (Fig. B) and 500 mg q24h (Fig. 1D) showed that reducing the dosing interval had a significant impact on levofloxacin bactericidal activity, with the more frequent administration (three times daily) being the more effective treatment simulated for a daily dose of 500 mg. These data could not be predicted or explained by any one of the three PK/PD indices because the $fAUC_{0-24h}$/MIC was the same for all treatments (30 h), the fC_{max}/MIC was bigger and the $T > MIC$ was longer for the single daily dose, which was shown to be the least effective of the three dosing regimens simulated. The explanation for this effect is unclear so far.

These results indicate that it does make a difference if a total daily dose is given once, twice or three times a day, although the *f*AUC/MIC is the same. The same observation was made by Sánchez Navarro et al. [31], for instance, after comparing ciprofloxacin standard dosage regimens (250 mg/12 h versus 500 mg/24 h) for the treatment of urinary tract infections. Since the $fAUC_{0-24h}$ was the same for the same daily dose, both regimens should present similar bactericidal effect. However, the 500 mg/24 h dosing regimen

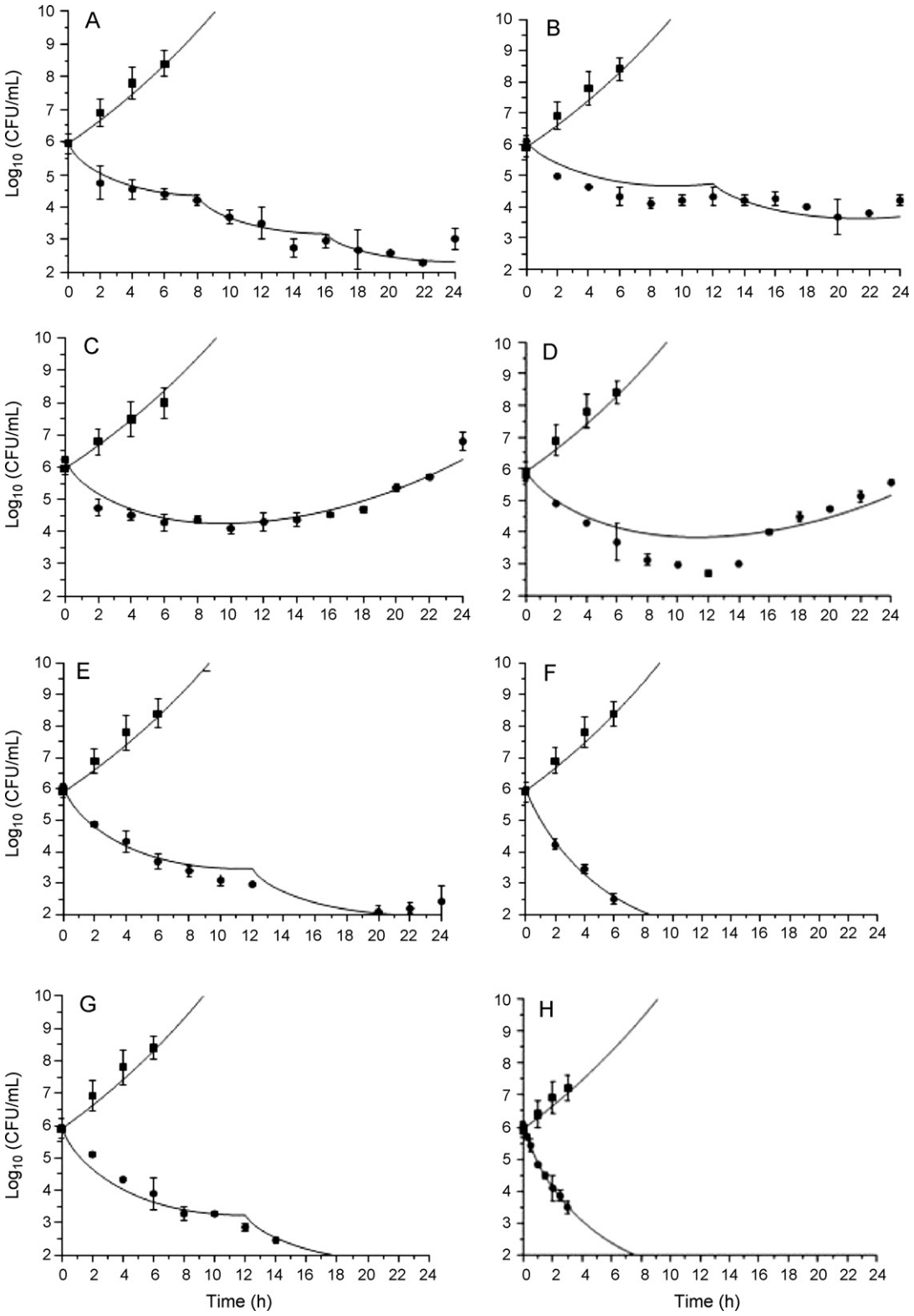


Fig. 1. Levofloxacin fitted time–kill curves following (A) 167 mg every 8 h, (B) 250 mg every 12 h (q12h), (C) 250 mg every 24 h (q24h), (D) 500 mg q24h, (E) 375 mg q12h, (F) 750 mg q24h, (G) 500 mg q12h and (H) 1000 mg q24h. ■, Control without drug; ●, treated group. $n = 3$ experiments/group. Errors bars indicate standard deviation. CFU, colony-forming units.

produced a more favourable antimicrobial profile in their study [31].

When increasing the single daily dose of levofloxacin from 500 mg to 750 mg or to 1000 mg the drug was found to be more effective against *S. pneumoniae* owing to higher exposures that resulted in $f\text{AUC}_{0-24h}$/MIC ratios of 45 and 60, respectively. These results agree with those reported by Lister [17] who suggested that when the levofloxacin $f\text{AUC}_{0-24h}$/MIC ratio falls below 30, the likelihood of bacterial eradication is diminished. Fractionating these higher total daily doses resulted in less effective treatments as can be seen in the time–kill curves after 375 mg q12h (Fig. 1E) or 500 mg q12h (Fig. 1G) as well as in the values of k_{max} reported in Table 1,

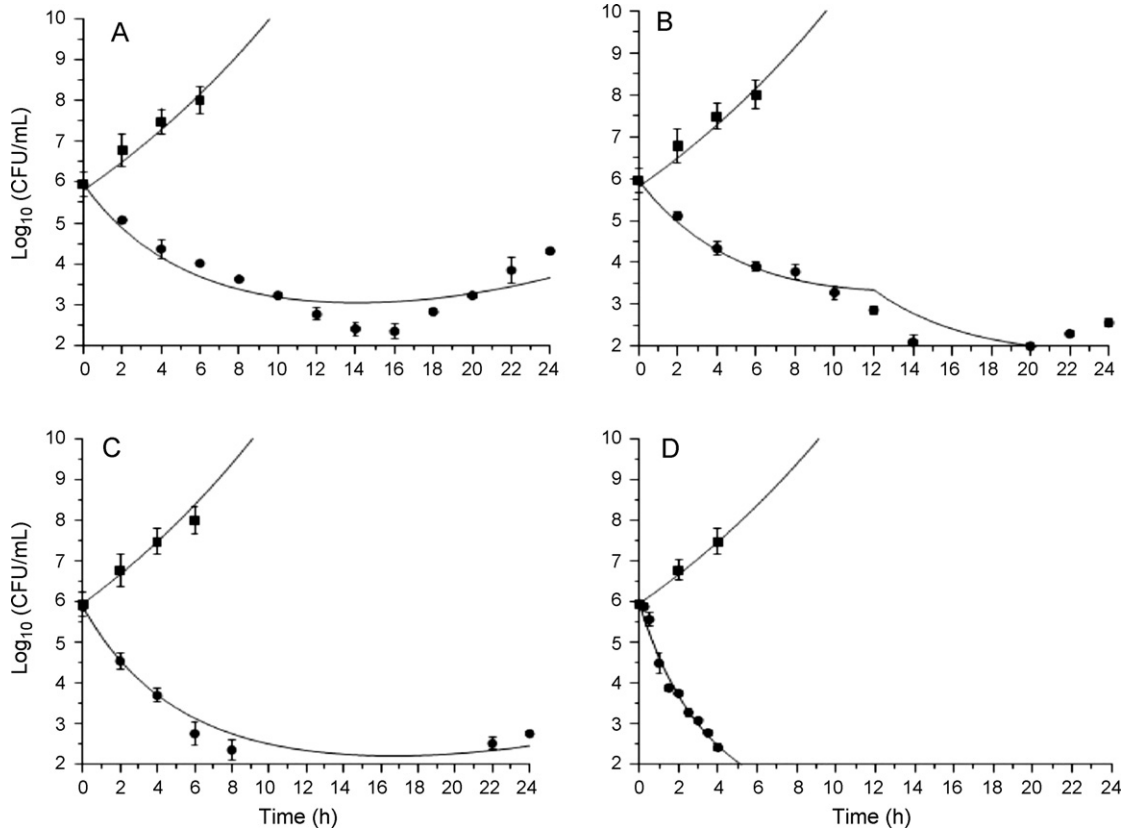


Fig. 2. Gatifloxacin fitted time–kill curves following (A) 50 mg every 24 h (q24h), (B) 50 mg every 12 h, (C) 100 mg q24h and (D) 200 mg q24h. ■, Control without drug; ●, treated group. $n = 3$ experiments/group. Errors bars indicate standard deviation. CFU, colony-forming units.

although a reduction of >3 log CFU was observed for these regimens in the time interval evaluated. In this case it seems that the fC_{max}/MIC index was a better predictor of infection outcome rather than fAUC$_{0-24h}$/MIC. These findings corroborate those obtained by Noreddin et al. [32], which concluded that a daily dose of 750 mg of levofloxacin leads to a higher probability of PD target attainment and improved bacteriological outcome against *S. pneumoniae* in patients with CAP, in addition to decreasing the length of therapy.

Gatifloxacin recommended clinical dosing regimens were a 200 mg or 400 mg daily dose. The dose range evaluated in this study was 50–400 mg/day. For the 400 mg daily dose, the killing effect was so pronounced that it was not possible to count bacteria in the model after 2 h of experiment (data not shown). The 50 mg q12h, 100 mg q24h and 200 mg q24h dosing regimens simulated in vitro produced >3 log reduction in CFU in 24 h (Fig. 2).

Gatifloxacin 50 mg q12h and 50 mg q24h, which are dosing regimens with a peak concentration of 0.35 mg/L, lower than the drug MIC for *S. pneumoniae*, produced CFU reduction for a period longer than 12 h (Fig. 2). This suggests that gatifloxacin subinhibitory concentrations produce a bactericidal effect and confirm that the MIC

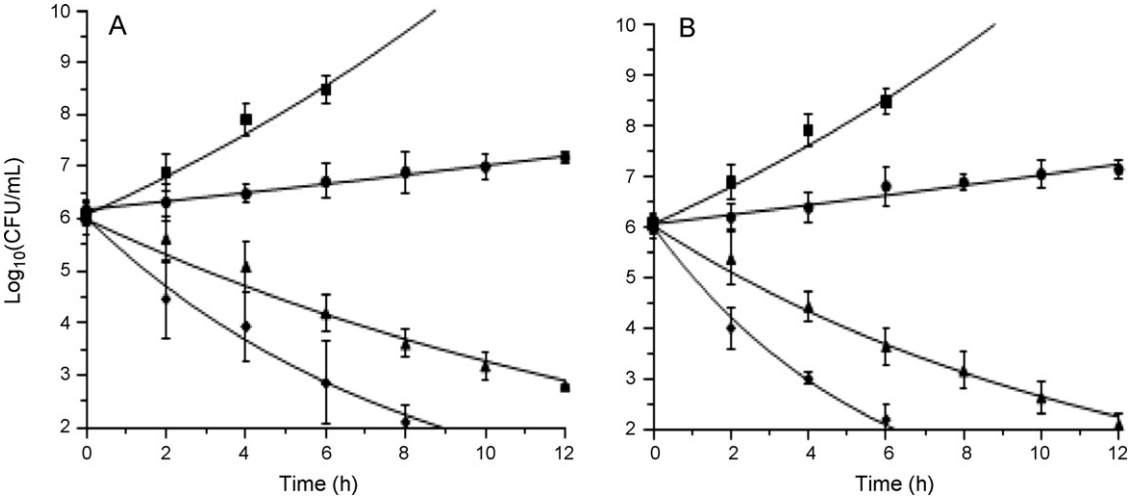


Fig. 3. Levofloxacin (A) and gatifloxacin (B) time–kill curves for constant concentrations of multiples of the respective minimum inhibitory concentration (MIC). MICs were 1 mg/L for levofloxacin and 0.5 mg/L for gatifloxacin. ■, Control without drug; ●, 0.5× MIC; ▲, 1.0× MIC; ♦, 2.0× MIC. $n = 3$ experiments/group. Errors bars indicate standard deviation. CFU, colony-forming units.

is a threshold value that has to be viewed with care. For all regimens investigated except 200 mg q24h, bacteria re-growth was observed by the end of the experimental period. The effective 200 mg q24h and 400 mg q24h were the only gatifloxacin regimens with an $fAUC_{0-24h}$/MIC ratio close to or greater than 30. In this context, these results support the recommended dose of gatifloxacin 200 mg or 400 mg q24h as regimens effective for *S. pneumoniae* eradication and suggest that the $fAUC_{0-24h}$/MIC ratio is a good predictor of infection outcome for this fluoroquinolone.

In the experiments where constant multiples of the MIC were simulated, the time–kill curves for both fluoroquinolones showed a large difference in the CFU count after 12 h when comparing the effect of concentrations representing 0.5× MIC and 1× MIC (Fig. 3). Whilst a concentration equivalent to 0.5× MIC only slowed down bacterial growth, concentrations similar to the MIC or 2× MIC actually resulted in killing, with the higher concentrations producing bacterial eradication after 6 h or 8 h for gatifloxacin and levofloxacin, respectively. Although gatifloxacin was apparently more efficacious and potent when comparing both drugs for the same MIC multiple, neither the EC_{50} nor the k_{max} averages were statistically different ($\alpha = 0.05$). Comparison of these parameters with those obtained for each drug after fluctuating concentrations were not statistically different either. These results indicate that if these fluoroquinolones were used as constant-rate infusion, concentrations equivalent to twice the bacterial MIC would be effective.

The results obtained using the in vitro model to build the time–kill curves and subsequently modelling with an E_{max} PK/PD model agreed with the results reported from clinical studies and allowed for a better comparison of dosing regimens for these fluoroquinolones. The results also indicate that the target values of the PK/PD indices fC_{max}/MIC and $fAUC_{0-24h}$/MIC cannot be universally applied to predict bacterial eradication for either one of the drugs investigated besides their inability to serve as a guide to compare drugs in the same antimicrobial class.

5. Conclusions

The PK/PD E_{max} model employed adequately described levofloxacin and gatifloxacin time–kill curves when fluctuating or constant concentration were simulated against *S. pneumoniae* in an in vitro model of infection generating parameters that allowed for comparison of the potency and efficacy of these fluoroquinolones following different dosing regimens. The model can be used for simulating regimens not investigated and for optimising both drug regimens to treat streptococcal infections.

Acknowledgments

The authors thank Bristol-Myers Squibb and Cristalia for supplying gatifloxacin and levofloxacin, respectively.

Funding: This work was financially supported by CNPq (Conselho Nacional de Pesquisa e Desenvolvimento, Brazil) (project # 473317/2004-4).

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Woodhead M. Community-acquired pneumonia. In: Franklin J, editor. Horizons in medicine 16. London, UK: Royal College of Physicians; 2004.
[2] Colice GL, Morley MA, Asche C, Birnbaum HG. Treatment costs of community-acquired pneumonia in an employed population. Chest 2004;125:2140–5.
[3] Mandell LA, Bartlett JG, Dowel SF, File Jr TM, Musher DM, Whitney C, et al. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin Infect Dis 2003;37:1405–33.
[4] Iochimescu OC, Iochimescu AG, Iannini PB. Severity scoring in community-acquired pneumonia caused by *Streptococcus pneumoniae*: a 5-year experience. Int J Antimicrob Agents 2004;24:485–90.
[5] File Jr TM. Clinical implications and treatment of multiresistant *Streptococcus pneumoniae* pneumonia. Clin Microbiol Infection 2006;12(Suppl. 3):31–41.
[6] File Jr TM. Antimicrobial therapy of community-acquired pneumonia. Infect Dis Clin North Am 2004;18:993–1016, xi.
[7] Wimer SM, Garrison MW. Levofloxacin: a therapeutic review. Clin Ther 1998;20:1049–70.
[8] Müller JL. Oral and i.v. formulations of gatifloxacin cleared for U.S. market. Am J Health Sys Pharm 2000;57:204.
[9] Blondeau JM. Expanded activity and utility of the new fluoroquinolones: a review. Clin Ther 1999;21:3–40.
[10] Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998;26:1–12.
[11] Zhanel GG, Ennis K, Vercaigne L, Walkty A, Gin AS, Embil J, et al. A critical review of the fluoroquinolones: focus on respiratory infections. Drugs 2002;62:13–59 [Erratum in: Drugs 2002;62:944].
[12] Zhanel GG, Noreddin AM. Pharmacokinetics and pharmacodynamics of the new fluoroquinolones: focus on respiratory infections. Curr Opin Pharmacol 2001;1:459–63.
[13] Ambrose PG, Grasela DM, Grasela TH, Passarell J, Mayer HB, Pierce PF. Pharmacodynamics of fluoroquinolones against *Streptococcus pneumoniae* in patients with community-acquired respiratory tract infections. Antimicrob Agents Chemother 2001;45:2793–7.
[14] Dalhoff A, Schmitz FJ. In vitro antibacterial activity and pharmacodynamics of new quinolones. Eur J Clin Microbiol Infect Dis 2003;22:203–21.
[15] Andes D, Anon J, Jacobs MR, Craig WA. Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. Clin Lab Med 2004;24:477–502.
[16] Ebisu H, Kishil R, Takei M, Fukudu H. The effect of pharmacokinetic/pharmacodynamic (PK/PD) parameters of gatifloxacin on its activity and resistance selectivity against clinical isolates of *Streptococcus pneumoniae*. J Infect Chemother 2003;9:210–4.
[17] Lister PD. Pharmacodynamics of gatifloxacin against *Streptococcus pneumoniae* in an in vitro pharmacokinetic model: impact of area under the curve/MIC ratios on eradication. Antimicrob Agents Chemother 2002;46:69–74.
[18] Rodvold KA, Neuhauser M. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Pharmacotherapy 2001;21:233S–52S.
[19] Dudley MN, Ambrose PG. Pharmacodynamics in the study of drug resistance and establishing in vitro susceptibility breakpoints: ready for prime time. Curr Opin Microbiol 2000;3:515–21.
[20] Liu P, Rand KH, Obermann B, Derendorf H. Pharmacokinetic–pharmacodynamic modelling of antibacterial activity of cefpodoxime and cefixime in in vitro kinetic models. Int J Antimicrob Agents 2005;25:120–9.
[21] Zeitlinger MA, Dehghanyar P, Mayer BX, Schenk BS, Neckel U, Heinz G, et al. Relevance of soft-tissue penetration by levofloxacin for target site bacterial killing in patients with sepsis. Antimicrob Agents Chemother 2003;47:3548–53.
[22] Bellmann R, Kuchling G, Dehghanyar P, Zeitlinger M, Minar E, Mayer BX, et al. Tissue pharmacokinetics of levofloxacin in human soft tissue infections. Br J Clin Pharmacol 2004;57:563–8.
[23] Hutschala D, Skhirtladze K, Zuckermann A, Wisser W, Jaksch P, Mayer-Helm BX, et al. In vivo measurement of levofloxacin penetration into lung tissue after cardiac surgery. Antimicrob Agents Chemother 2005;49:5107–11.
[24] Tasso L, Bettoni CC, Oliveira L, Dalla Costa T. Evaluation of gatifloxacin penetration into skeletal muscle and lung by microdialysis in rats. Int J Pharm 2008;358:96–101.
[25] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 6th ed. Document M7-A6. Wayne, PA: CLSI; 2003.
[26] Zhang X, Overholser BR, Kays MB, Sowinski KM. Gatifloxacin pharmacokinetics in healthy men and women. J Clin Pharmacol 2006;46:1154–62.
[27] Zhanel GG, Walkty A, Vercaigne L, Karlowsky JA, Embil J, Gin AS, et al. The new fluoroquinolones: a critical review. Can J Infect Dis Med Microbiol 1999;10:207–38.
[28] Delacher S, Derendorf H, Hollenstein U, Brunner M, Joukhadar C, Hofmann S, et al. A combined in vivo pharmacokinetic–in vitro pharmacodynamic approach to simulate target site pharmacodynamics of antimicrobials in humans. J Antimicrob Chemother 2000;46:733–9.
[29] Schuck EL, Dalhoff A, Stass H, Derendorf H. Pharmacokinetic/pharmacodynamic (PK/PD) evaluation of a once-daily treatment using ciprofloxacin in an extended-release dosage form. Infection 2005;33(Suppl. 2):22–8.
[30] Palma EC, Dalla Costa T. PK/PD modeling of the bactericidal effect of norfloxacin against different microorganisms. Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Rio Grande do Sul; 2003 [academic dissertation; in Portuguese].
[31] Sánchez Navarro MD, Sayalero Marinero ML, Sánchez Navarro A. Pharmacokinetic/pharmacodynamic modelling of ciprofloxacin 250 mg/12 h versus 500 mg/24 h for urinary infections. J Antimicrob Chemother 2002;50:67–72.
[32] Noreddin AM, Marras TK, Sanders K, Chan CK, Hoban DJ, Zhanel GG. Pharmacodynamic target attainment against *Streptococcus pneumoniae* using levofloxacin 500 mg, 750 mg and 1000 mg once daily in plasma (P) and epithelial lining fluid (ELF) of hospitalized patients with community-acquired pneumonia (CAP). Int J Antimicrob Agents 2004;24:479–84.